Clinical Practice: Original Paper

nephron Clinical Practice

Nephron 2019;141:188–200
DOI: 10.1159/000495726

Received: MAY 24, 2018
Accepted After Revision: November 22, 2018
Published online: January 9, 2019

Mean Corpuscular Volume and Mortality in Incident Hemodialysis Patients

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Keywords
Mean corpuscular volume · Incident hemodialysis · Mortality · Anemia

Abstract
Background/Aims: Anemia is common in patients with advanced chronic kidney disease (CKD). A proportion of patients present with macrocytic anemia, manifested by elevated mean corpuscular volume (MCV), which has been associated with worse outcomes in CKD patients. However, it is unknown whether elevated MCV is associated with higher mortality risk in incident hemodialysis (HD) patients. ***Methods:*** This retrospective observational cohort study examined all-cause, cardiovascular, and infectious mortality associations with both baseline and time-varying MCV in 109,501 incident HD patients using Cox proportional hazards models with 3 levels of hierarchical multivariable adjustment. Odds ratios of high versus low baseline MCV were evaluated using logistic regression. ***Results:*** The mean age of patients was 65 ± 15 (standard deviation) years and the cohort was 44% female, 58% diabetic, and 31% African American. Higher MCV was associated with older age, female sex, non-Hispanic White race-ethnicity, alcohol consumption, and having a decreased albumin or protein intake. Patients with higher MCV levels (>98 fL) had a higher all-cause, cardiovascular, and infectious mortality risk in both baseline and time varying models, and across all levels of adjustment. In the fully adjusted models, compared to a reference of MCV 92–<94 fL, patients with a baseline MCV >100+ fL had a 28% higher risk of all-cause mortality (hazard ratio [HR] 1.28, 95% CI 1.22–1.34), 27% higher risk of cardiovascular mortality (HR 1.27, 95% CI 1.18–1.36), and 18% higher risk of infectious mortality (HR 1.18, 95% CI 1.02–1.38). Associations of higher MCV with these adverse outcomes persisted across all exam-

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ined subgroups of clinical characteristics. ***Conclusions:*** Higher MCV was associated with higher all-cause, cardiovascular, and infectious mortality in HD patients. Further investigation is necessary to understand the underlying nature of the observed association.

Introduction

Anemia is common in patients with advanced chronic kidney disease (CKD) with a prevalence of about 53% in CKD stage 5 [1]. Erythrocyte size in anemia can be differentially diagnosed into microcytic, normocytic, or macrocytic anemia by the index of mean corpuscular volume (MCV) [2]. Anemia in CKD patients is usually normocytic and normochromic [3], characterized by reduced erythropoietin production [4], and is associated with reduced red blood cell (RBC) survival [5]. Some patients with advanced CKD present with macrocytosis [3]. Elevated MCV (generally 100+ fL; macrocytic anemia) is often characteristic of underlying conditions such as nutritional deficiencies, drug use, or primary bone marrow disorders [2, 6–8], and has been associated with worse outcomes in patients with acute decompensated heart failure [9] and patients undergoing coronary intervention [10].

In a recent study investigating a small cohort of 1,439 Taiwanese patients with stages 3–5 CKD, Hsieh et al. [11], found that over a median of 1.9 years of follow-up, patients with higher MCV (defined as greater than the median value of 90.8 fL) had a higher risk of all-cause, cardiovascular, and infection-related mortality, compared to patients with MCV levels below the median. Additionally, an earlier prospective, single-center study including 150 prevalent Canadian dialysis patients found an association between higher MCV (defined as >102 fL) and mortality over 9 months of follow-up [12]. However, whether higher MCV is associated with mortality in incident hemodialysis (HD) patients is still unknown. We hypothesized that in a large nationally representative cohort of incident HD patients in the United States, higher MCV levels would be associated with a higher risk of mortality.

Methods

Study Population and Data Source

We examined the data from a total of 208,820 patients with end stage renal disease (ESRD) who initiated dialysis therapy from January 1, 2007 to December 31, 2011 within a large dialysis care organization in the United States. This cohort has been previously described [13]. We excluded 46,156 patients who had <60 days of total treatment during their total follow-up time, 29,502 for receiving treatment with a modality other than in-center HD, 21,145 for not having treatment data in the first patient quarter (91 days from patient first dialysis date), and 2,516 for not having MCV data in the first patient quarter. The final analytical cohort consisted of 109,501 incident HD patients (online suppl. Fig. S1; for all online suppl. material, see www.karger.com/doi/10.1159/000495726).

Additionally, we created 3 subcohorts for sensitivity analysis where we further restricted the analytical cohort based on the availability of the covariate of interest: (i) we excluded 100,208 incident HD patients for missing folate in the first patient quarter, which led to a subcohort of 9,293 patients with averaged MCV and folate measurements in the first patient quarter, (ii) for the second subcohort, 98,810 incident HD patients were excluded for missing vitamin B12 (B12) measurements in the first patient quarter, which resulted in a total of 12,691 patients with MCV and B12 measurements in the first patient quarter, and finally (iii) 73,167 patients were excluded for missing residual renal urea clearance (KRU) values in the first patient quarter, and therefore, the third subcohort consisted of 36,334 incident HD patients.

All data were obtained from electronic records of the dialysis organization. ICD-9 codes listed in patient electronic medical records were used to determine the following conditions: alcohol abuse, arteriosclerotic heart disease (ASHD), cerebrovascular disease, congestive heart failure (CHF), chronic obstructive pulmonary disease (COPD), diabetes mellitus, dyslipidemia, history of cancer, hypertension, liver disease, human immunodeficiency virus (HIV), other cardiovascular disease, and substance abuse. Blood samples were drawn using standardized techniques in all dialysis clinics and were transported to a central laboratory in Deland, Florida, typically within 24 hours. All laboratory values were measured using automated and standardized methods. Serum creatinine, phosphorus, calcium, albumin, bicarbonate, total iron-binding capacity (TIBC), and red cell distribution width were measured monthly. Serum intact parathyroid hormone (iPTH) and ferritin were measured at least quarterly. Hemoglobin was measured weekly to biweekly in most patients. Delivered dialysis dose was estimated by single-pool Kt/V (spKt/V) using the urea kinetic model [14]. Body mass index (BMI) was calculated as post-HD session body weight in kilograms divided by height in meters squared. MCV was routinely reported in femtoliters (fL, or 10^{-15} L), along with complete blood cell count, and was calculated by dividing the Hematocrit by the RBC count as follows: MCV (fL) = (Hematocrit [%]/RBC [in million per μL]).

To minimize variability, variables with repeated measures within each 3-month period (91-day intervals) were averaged to obtain a single quarterly mean value. Measurements taken during the first 91 days on dialysis therapy, referred to as the "first patient quarter," were used as baseline values.

The study was approved by the institutional review committees of the University of California, Irvine, and it was exempted from obtaining informed written consent from the patients due to its nonintrusive nature and also because patients wanted to remain anonymous.

Exposure and Outcome Ascertainment

MCV was the primary exposure of interest. All-cause, cardiovascular, and infectious mortality were examined as the primary and secondary outcomes, respectively. For the definition of cardio-

vascular and infectious mortality, please refer to online supplementary Table S1a and b. Patients were divided into 9 groups of MCV spaced into equal intervals of 2 fL: <86, 86–<88, 88–<90, 90–<92, 92–<94 (reference), 94–<96, 96–<98, 98–<100, and 100+. Associations of MCV with mortality outcomes were examined in both baseline and time-varying models. Patients were followed from the first date of HD treatment until the patients were censored through one of the following events: death, kidney transplantation, transfer to another dialysis provider, or end of the study period.

Statistical Analyses

Baseline patient characteristics were summarized across baseline MCV groups using proportions, mean (± standard deviation [SD]) for parametric variables, or median (interquartile range) for non-parametric variables, and were compared using tests-for-trend. Odds ratios (OR) for high (MCV ≥93 fL) vs. low (MCV <93 fL) baseline MCV were calculated using logistic regression. Linear regression and correlation coefficients (Spearman or Pearson as appropriate) were also calculated to evaluate serum laboratory values and medication dosage associations with baseline MCV.

To illustrate trajectories of quarterly averaged MCV across 20 patient quarters, we used a mixed-effects regression model and stratified trajectories by the baseline MCV groups, baseline median weekly erythropoiesis stimulating agent (ESA) dose groups, or baseline cumulative monthly intravenous (IV) iron dose groups. The cut points for the median weekly ESA dose groups and cumulative monthly IV iron dose groups were data derived. We further performed time-varying adjustment for weekly median ESA dose and cumulative monthly IV iron dose in the MCV baseline stratified model, weekly median ESA dose in the baseline ESA stratified model, and monthly cumulative IV iron dose in the baseline IV iron stratified model.

Associations of MCV with all-cause, cardiovascular, and infectious mortality outcomes were estimated using Cox proportional hazards models for both baseline and time-varying models. The proportionality assumption was checked using plots of log (–log [survival rate]) against log (survival time). We additionally explored potentially non-linear relationships between MCV and mortality outcomes using restricted cubic spline models with 4 knots placed at the 5th, 35th, 65th, and 95th percentile values of MCV. Mortality associations of high vs. low MCV were also examined across a priori selected subgroups in fully adjusted models, and were tested for interactions using the Wald test. In sensitivity analyses, we used competing risk regression to further explore the association between MCV and cardiovascular and infectious mortality, with cardiovascular or infectious death as the event of interest and non-cardiovascular or non-infectious mortality as the competing event, respectively.

All models were examined across 3 levels of hierarchical multivariable adjustment. The first level was unadjusted and included only MCV as the exposure. The second level was case-mix adjusted, which additionally included demographic data (age and sex), race-ethnicity (non-Hispanic White, African American, Hispanic, Asian, and other), comorbid conditions (alcoholism, ASHD, cerebrovascular disease, CHF, COPD, diabetes mellitus, dyslipidemia, history of cancer, hypertension, liver disease, other cardiovascular disease, and substance abuse), insurance type (Medicare, Medicaid, other), HD access type (central venous catheter, arteriovenous fistula, arteriovenous graft, unkown, other), and spKt/V. The third level (fully adjusted model) added markers of the malnutrition-inflammation complex syndrome (MICS), for which we used 17 surrogates of nutritional and inflammatory status: hemoglobin, albumin, calcium, phosphorus, iPTH, iron saturation, TIBC, ferritin, bicarbonate, white blood cell count, lymphocyte percentage, creatinine, alkaline phosphatase (ALP), BMI, normalized protein catabolic rate (nPCR), cumulative monthly IV iron dose per quarter, and ESA quarterly median dose per week.

In logistic regression analyses, models were restricted to patients that had the risk factor of interest, and were adjusted for all other components in the fully adjusted model minus the exposure. In time-varying models, MCV, access type, laboratory values, and medication use were time-updated. In additional analyses, we adjusted for (i) folate, (ii) B12, and (iii) KRU individually, in order to evaluate potential confounding by these covariates in models restricted to patients with available data on these variables in the first patient quarter.

For most covariates (including laboratory markers), baseline data were <1% missing for the total cohort. Folate, B12, and KRU were missing at 92, 88, and 67%, respectively, and therefore were only included in sensitivity analyses. In time-varying analyses, MCV values were carried forward until subsequent updated values were reported. Missing values were handled by imputation by median for quantitative variables, or by creation of a missing category for categorical data. All analyses were implemented using Stata, version 13.1 (Stata Corporation, College Station, TX, USA).

Results

Study Population

The analytical cohort included 109,501 HD patients and total cohort median (interquartile range) follow-up time was 494 (230–922) days. Baseline characteristics stratified by the 9 MCV categories are shown in Table 1. Mean ± SD age of the cohort was 63 ± 15 years. The cohort was comprised of 44% females, 58% diabetic patients, and 31% African American patients.

At baseline, patients with higher MCV were more likely to be female, older, non-diabetic, non-Hispanic White, on Medicare, and have comorbidities of alcoholism, COPD, history of cancer, and liver disease. Patients with lower MCV were more likely to have comorbidities of CHF and hypertension. At higher levels of MCV, calcium, ferritin, folate, serum iron, iron saturation, and spKt/V increased while albumin, creatinine, iPTH, KRU, lymphocyte, phosphorous, and total iron binding capacity decreased. Patients with higher levels of MCV were also more likely to receive lower doses of IV iron.

Predictors of Higher MCV

Odds ratios of having MCV levels above the median (≥93 fL) in 109,501 HD patients are shown in Table 2 for unadjusted, case-mix adjusted, and fully adjusted (case-mix plus MICS adjusted) models. In fully adjusted mod-

Table 1. Baseline characteristics of 109,501 patients by baseline MCV level

	Total cohort	MCV, fL									*p* value
		<86	86–<88	88–<90	90–<92	92–<94	94–<96	96–<98	98–<100	100+	
n (%)	109,501	12,029 (11)	8,441 (8)	12,032 (11)	14,884 (14)	15,983 (15)	14,490 (13)	11,366 (10)	8,081 (7)	12,195 (11)	
Age, years	63±15	57±15	58±15	59±15	61±15	63±15	64±15	66±14	67±14	69±14	<0.001
Gender, female, %	44	38	39	40	42	43	46	47	49	49	<0.001
Diabetes, %	58	65	64	63	61	59	57	55	53	48	<0.001
BMI, kg/m^2	28.1±7.3	29.3±7.5	28.9±7.4	28.8±7.3	28.3±7.3	27.9±7.1	27.8±7.2	27.6±7.3	27.2±7.2	27.0±7.3	<0.001
Race-Ethnicity, %											
Asian	3	4	3	3	3	3	3	4	3	4	0.973
African American	31	55	41	35	30	28	26	25	24	22	<0.001
Non-Hispanic White	47	26	36	41	45	48	51	54	57	62	<0.001
Hispanic	15	11	16	17	18	17	16	14	13	10	<0.001
Other	4	4	4	4	4	4	4	3	3	3	<0.001
Insurance, %											
Medicare	54	48	48	49	51	53	55	57	60	62	<0.001
Medicaid	7	8	8	8	8	7	7	6	5	5	<0.001
Other	39	43	43	42	41	40	38	38	35	33	<0.001
Comorbid conditions, %											
Alcohol	0.24	0.17	0.18	0.2	0.24	0.23	0.19	0.33	0.26	0.37	<0.001
ASHD	14	15	14	15	14	14	14	14	15	15	0.732
CBVD	1.8	1.8	1.7	1.6	1.8	1.8	1.8	1.9	2	1.8	0.063
CHF	37	39	38	38	36	36	36	36	37	36	<0.001
COPD	5.1	4.4	4.3	4.6	4.8	5.0	5.1	5.4	6.3	6.3	<0.001
Dyslipidemia	25	26	25	25	25	25	25	25	25	25	0.358
History of cancer	2.32	1.65	1.97	1.77	2.18	2.53	2.51	2.54	2.82	2.85	<0.001
HIV	0.5	0.5	0.4	0.4	0.4	0.4	0.4	0.5	0.7	0.9	<0.001
Hypertension	51	55	52	52	50	51	51	50	51	50	<0.001
Liver disease	1.49	1.36	1.07	1.16	1.39	1.36	1.46	1.55	1.91	2.19	<0.001
Other cardiac disease	15	15	14	15	14	15	15	15	17	16	<0.001
Access type, %											
AVF	15	16	15	15	15	15	15	15	15	15	0.350
AVG	4	5	4	4	4	4	4	4	4	4	0.582
CVC	75	73	75	75	76	76	75	74	75	73	0.103
Other	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.341
Unknown	6	6	6	6	6	6	6	6	6	7	<0.001
Serum laboratory values											
Albumin, g/dL	3.51±0.48	3.54±0.48	3.52±0.48	3.52±0.48	3.52±0.48	3.51±0.47	3.51±0.47	3.49±0.48	3.48±0.48	3.46±0.49	<0.001
ALP, U/L	87.0 (68.8–115.0)	86.6 (68.8–113.7)	87.3 (69.3–113.0)	87.0 (69.3–113.0)	87.0 (68.5–114.8)	86.7 (68.7–114.5)	86.5 (68.5–114.3)	87.0 (68.3–115.0)	87.7 (68.7–118.0)	88.8 (69.0–120.3)	<0.001
Bicarbonate, mEq/L	23.6±2.7	23.7±2.6	23.6±2.6	23.6±2.6	23.5±2.7	23.6±2.7	23.5±2.7	23.6±2.8	23.6±2.8	23.6±2.9	
Calcium, mg/dL	9.10±0.56	9.08±0.56	9.08±0.55	9.07±0.56	9.08±0.55	9.08±0.56	9.09±0.57	9.11±0.57	9.13±0.57	9.15±0.57	<0.001
Creatinine, mg/dL	5.9±2.4	6.4±2.5	6.2±2.5	6.1±2.5	6.0±2.4	5.9±2.4	5.7±2.3	5.6±2.2	5.5±2.1	5.3±2.0	<0.001

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Table 1. (continued)

	Total cohort	MCV, fL									*p* value
		<86	86–<88	88–<90	90–<92	92–<94	94–<96	96–<98	98–<100	100+	
Ferritin, ng/mL	283 (164–485)	230 (126–409)	250 (142–430)	258 (149–442)	273 (161–464)	283 (166–476)	298 (176–508)	306 (183–516)	321 (189–542)	341 (202–585)	<0.001
Folate, ng/mL	10.2±4.3	9.9±4.2	9.9±4.2	9.6±4.2	10.2±4.2	10.2±4.3	10.4±4.3	10.4±4.3	10.7±4.5	10.5±4.5	<0.001
Hemoglobin, g/dL	11.1±1.2	10.9±1.2	11.1±1.2	11.1±1.2	11.2±1.2	11.2±1.2	11.1±1.2	11.2±1.2	11.2±1.2	11.1±1.2	<0.001
iPTH, pg/mL	313 (197–486)	354 (228–535)	332 (214–506)	326 (209–503)	318 (204–486)	315 (198–484)	307 (190–478)	301 (186–476)	293 (182–460)	273 (168–435)	<0.001
Iron, μg/dL	50.5±20.1	44.9±18.4	46.6±18.8	47.6±18.3	49.3±19.1	51.1±19.0	51.6±20.2	52.7±20.0	54.0±20.8	58.0±23.3	<0.001
Iron saturation, %	23.1±9.1	20.0±8.1	20.9±8.1	21.5±8.1	22.3±8.2	22.8±8.2	23.6±8.9	24.4±9.1	25.1±9.4	27.5±11.6	<0.001
KRU, mL/min	3.27 (1.69–5.49)	3.63 (1.92–6)	3.52 (1.91–5.88)	3.47 (1.83–5.77)	3.40 (1.77–5.67)	3.20 (1.69–5.42)	3.18 (1.66–5.33)	3.11 (1.59–5.18)	2.99 (1.54–5.0)	2.88 (1.4-4.9)	<0.001
LDH, U/L	199 (71–235)	200 (86–234)	198 (83–234)	200 (89–235)	199 (79–234)	199 (79–234)	198 (78–235)	199 (89–236)	201 (71–236)	202 (74–241)	<0.001
Lymphocyte (% of WBC)	20.7±7.5	21.6±7.5	21.0±7.3	20.9±7.3	20.6±7.2	20.5±7.3	20.5±7.5	20.4±7.6	20.3±7.6	20.4±8.4	<0.001
nPCR, g/kg/day	0.79±0.22	0.79±0.21	0.79±0.21	0.79±0.22	0.80±0.22	0.79±0.22	0.80±0.22	0.78±0.22	0.79±0.22	0.78±0.22	<0.001
Phosphorus, mg/dL	4.92±1.15	5.01±1.12	5.03±1.16	5.00±1.14	4.98±1.16	4.94±1.16	4.90±1.16	4.86±1.15	4.81±1.13	4.73±1.13	<0.001
RDW, %	16.3±1.7	17.2±2.0	16.3±1.7	16.2±1.6	16.1±1.6	16.1±1.6	16.1±1.6	16.2±1.6	16.3±1.7	16.8±1.9	<0.001
Reticulocyte, %	2.7 (2.1–3.4)	2.7 (2.1–3.4)	2.8 (2.2–3.4)	2.8 (2.2–3.4)	2.8 (2.2–3.4)	2.7 (2.1–3.4)	2.7 (2.1–3.4)	2.6 (2.1–3.4)	2.7 (2.1–3.5)	2.6 (2.0–3.4)	0.006
spKt/V	1.46±0.33	1.41±0.33	1.43±0.32	1.45±0.33	1.46±0.33	1.47±0.31	1.49±0.33	1.49±0.32	1.51±0.33	1.51±0.32	<0.001
TIBC, mg/dL	225±49	231±49	228±48	227±48	226±48	225±48	224±49	221±49	221±49	221±50	<0.01
Vitamin B12, pg/mL	620 (447–880)	627 (460–892)	596 (448–859)	587 (432–829)	601 (435–867)	599 (436–858)	626 (449–859)	636 (461–921)	651 (447–917)	653 (459–936)	<0.001
WBC, $\times 10^3/\mu L$	7.8±2.7	7.7±2.6	7.8±2.5	7.9±2.5	7.8±2.5	7.9±2.6	7.9±2.7	7.8±2.7	7.8±2.6	7.7±3.2	<0.001
Medications											
ESA Use, U/week	4,708 (1,500–11,957)	4,872 (1,540–12,168)	4,600 (1,500–11,896)	4,618 (1,466–11,880)	4,677 (1,500–11,661)	4,694 (1,500–11,917)	4,652 (1,467–11,818)	4,793 (1,513–12,168)	4,767 (1,523–11,822)	4,734 (1,508–12,100)	0.832
IV iron, mg/month	1,000 (400–1,400)	1,000 (500–1,525)	1,000 (500–1,500)	1,000 (500–1,500)	1,000 (425–1,450)	1,000 (450–1,400)	1,000 (400–1,400)	1,000 (400–1,400)	900 (350–1,400)	800 (250–1,300)	<0.001

Data are presented as means ± standard deviations, medians (interquartile ranges), or percentages, and compared across groups with tests for trend.

BMI, body mass index; ASHD, arteriosclerotic heart disease; CBVD, cerebrovascular disease; CHF, congestive heart failure; COPD, chronic obstructive pulmonary disease; HIV, human immunodeficiency virus; AVF, arteriovenous fistula; AVG, arteriovenous graft; CVC, central venous catheters; ALP, alkaline phosphatase; iPTH, intact parathyroid hormone; KRU, residual renal urea clearance; LDH, lactic acid dehydrogenase; nPCR, normalized protein catabolic rate; RDW, red cell distribution width; spKt/V, single-pooled Kt/V; TIBC, total iron binding capacity; WBC, white blood cell count; ESA, erythropoiesis stimulating agent; IV, intravenous; MCV, mean corpuscular volume.

els, odds of higher baseline MCV were associated with older age, female sex, alcohol consumption, COPD, and higher ALP. Conversely, being diabetic, being not of non-Hispanic White race-ethnicity (in particular African American race-ethnicity), having higher albumin, or having higher nPCR, was associated with lower likelihood of having a baseline MCV above the median.

Linear regression analysis and correlations showed similar results (online suppl. Table S2). MCV had positive correlations with values for ALP, B12, calcium, ferritin, folate, hemoglobin, serum iron, iron saturation, and spKt/V. It had negative correlations with albumin, creatinine, iPTH, KRU, lymphocyte, nPCR, phosphorous, red cell distribution width, total iron binding capacity, white blood cell count, and IV iron. However, the correlations between MCV and various laboratory variables were very weak. The strongest correlation was found between MCV and iron saturation. With every percent increase in iron saturation, MCV increased by 0.15 fL (correlation coefficient and p value, 0.23 and <0.001).

MCV Trajectories over 20 Patient Quarters

Overall, patients showed a gradual increasing trend toward higher MCV levels over 20 patient quarters. The hierarchical order of the baseline MCV groups was maintained, and the differences between groups remained relatively constant (online suppl. Fig. S2a). Adjustment for time-varying cumulative IV iron dose and median ESA dose did not substantially change the course of the trajectory (online suppl. Fig. S2b). Additionally, after stratifying patients according to their baseline ESA dose into 4 groups, MCV increased during 20 patient quarters and showed very little variance across ESA strata (online suppl. Fig. S2c). The trajectory remained consistent after adjusting for patient quarter median weekly ESA dose (online suppl. Fig. S2d). Likewise, we stratified patients into 4 strata for baseline cumulative monthly IV iron dose, and found no significant difference in mean MCV levels across iron strata over the course of 20 patient quarters (online suppl. Fig. S2e), which was not altered after adjustment for time-varying monthly cumulative IV iron dose (online suppl. Fig. S2f).

All-Cause Mortality

All-cause mortality rates increased linearly across the 9 groups of MCV and more than doubled from the group with the lowest MCV (<86 fL) to the group with the highest MCV (≥100 fL; 117 deaths versus 250 deaths per 1,000 person-years, online suppl. Table S3). In baseline unadjusted hazard ratio (HR) models, MCV was linearly associated with mortality risk (Fig. 1a), where patients with MCV ≥100 fL had a 65% higher risk of mortality compared to the referent (HR 1.65, 95% CI 1.58–1.72). After case-mix or case-mix plus MICS covariate adjustments, the protective benefit of lower MCV was attenuated, so patients with MCV below the referent had a similar mortality risk to the referent. In fully adjusted models, patients with high MCV maintained a higher mortality risk, with the highest risk for patients having MCV 100+ fL (HR 1.28, 95% CI 1.22–1.34).

In the 3 subcohorts including (i) 9,293 incident HD patients with baseline folate and MCV measurements, (ii) 12,691 incident HD patients with baseline B12 and MCV measurements, and (iii) 36,334 incident HD patients with baseline KRU and MCV measurements, the MCV-mortality association was similar to the main cohort in the case-mix plus MICS adjusted model. Further adjustment, separately for (i) folate, (ii) B12 and (iii) KRU, as shown in online supplementary Figure S3a–c, did not attenuate the association of higher MCV with mortality.

Time-varying models followed a similar pattern as the baseline MCV-mortality association. Higher MCV was associated with higher short-term risk of all-cause mortality across all levels of adjustment; however, lower MCV had no protective benefit compared to the referent (Fig. 1b, online suppl. Table S4). Restricted cubic splines modeling HRs for baseline (online suppl. Fig. S4a–c) and time-varying (online suppl. Fig. S5a–c) associations showed similar results and did not suggest the presence of nonlinear relationships between MCV and all-cause mortality.

Cardiovascular Mortality

Associations of MCV with cardiovascular mortality showed similar patterns to those in all-cause mortality models. In both baseline and time-varying models, the protective effect of lower MCV was attenuated by covariate adjustments (Fig. 1c, d, online suppl. Table S5, S6). Patients with higher MCV maintained a higher mortality risk across all levels of adjustment. In baseline fully adjusted models, patients with MCV 100+ fL had a 27% higher cardiovascular mortality risk compared to the referent (HR 1.27, 95% CI 1.18–1.36). Competing risk regression subhazard ratios (online suppl. Table S7) showed that the same patterns hold after accounting for non-cardiovascular mortality as a competing event.

Restricted cubic splines for baseline (online suppl. Fig. S4d–f) and time-varying (online suppl. Fig. S5d–f) cardiovascular mortality HRs did not suggest the presence of nonlinear relationships between MCV and cardiovascular mortality.

Table 2. Likelihood of having baseline MCV levels (≥93 fL) in 109,501 HD patients in the unadjusted, case-mix, and fully adjusted models

	Unadjusted			Case-mix			Case-mix + MICS		
	OR	95% CI	*p* value	OR	95% CI	*p* value	OR	95% CI	*p* value
Age, years (per 10)	1.36	1.35–1.37	<0.001	1.30	1.29–1.31	<0.001	1.34	1.33–1.36	<0.001
Gender, female	1.30	1.27–1.34	<0.001	1.30	1.27–1.34	<0.001	1.32	1.28–1.35	<0.001
Diabetes	0.71	0.70–0.73	<0.001	0.69	0.67–0.70	<0.001	0.72	0.70–0.74	<0.001
BMI, per 10 kg/m^2	0.80	0.79–0.81	<0.001	0.87	0.86–0.89	<0.001	0.93	0.91–0.95	<0.001
Race-Ethnicity									
Non-Hispanic White		1-referent			1-referent			1-referent	
Asian	0.73	0.69–0.79	<0.001	0.78	0.73–0.84	<0.001	0.70	0.65–0.75	<0.001
African American	0.46	0.44–0.47	<0.001	0.55	0.54–0.57	<0.001	0.53	0.51–0.55	<0.001
Hispanic	0.61	0.59–0.63	<0.001	0.77	0.74–0.80	<0.001	0.73	0.71–0.76	<0.001
Other	0.63	0.59–0.67	<0.001	0.75	0.70–0.81	<0.001	0.72	0.67–0.77	<0.001
Insurance									
Medicare		1-referent			1-referent			1-referent	
Medicaid	0.65	0.62–0.68	<0.001	0.99	0.95–1.05	0.840	0.97	0.92–1.02	0.221
Other	0.76	0.74–0.78	<0.001	0.93	0.91–0.96	<0.001	0.95	0.92–0.97	<0.001
Comorbid conditions									
Alcohol	1.22	0.95–1.55	0.114	1.48	1.14–1.91	0.003	1.31	1.01–1.71	0.045
ASHD	1.01	0.98–1.05	0.425	0.96	0.93–1.00	0.055	0.96	0.92–1.00	0.047
CBVD	1.05	0.96–1.15	0.253	0.97	0.88–1.07	0.527	0.98	0.89–1.08	0.671
CHF	0.93	0.91–0.95	<0.001	1.00	0.98–1.03	0.829	1.04	1.01–1.07	0.008
COPD	1.22	1.15–1.28	<0.001	1.03	0.97–1.10	0.281	1.10	1.04–1.17	0.002
Dyslipidemia	1.00	0.97–1.03	0.995	1.03	1.00–1.06	0.091	1.02	0.99–1.06	0.111
History of cancer	1.36	1.25–1.47	<0.001	1.08	0.99–1.17	0.069	1.00	0.91–1.08	0.917
Hypertension	0.95	0.93–0.97	<0.001	0.94	0.92–0.97	<0.001	0.95	0.93–0.98	<0.001
Liver disease	1.29	1.17–1.42	<0.001	1.34	1.21–1.50	<0.001	1.07	0.96–1.20	0.247
Other CD	1.08	1.04–1.11	<0.001	1.01	0.97–1.05	0.654	1.02	0.98–1.06	0.440
Access type									
CVC		1-referent			1-referent			1-referent	
AVF	1.01	0.98–1.04	0.616	0.96	0.92–0.99	0.012	0.93	0.90–0.97	<0.001
AVG	1.04	0.98–1.11	0.156	0.98	0.92–1.04	0.496	0.96	0.90–1.03	0.262
Other	0.90	0.61–1.32	0.589	0.97	0.65–1.44	0.874	0.95	0.63–1.42	0.785
Unknown	1.09	1.04–1.15	<0.001	1.11	1.05–1.17	<0.001	1.08	1.02–1.14	0.006
Serum laboratory values									
Albumin, per 0.5 g/dL	0.75	0.72–0.79	<0.001	0.79	0.75–0.83	<0.001	0.89	0.83–0.96	0.001
ALP, per 100 U/L	1.05	1.03–1.07	<0.001	1.11	1.09–1.13	<0.001	1.08	1.06–1.10	<0.001
B12, per 100 pg/mL	1.02	1.01–1.03	<0.001	1.02	1.01–1.03	<0.001	1.02	1.00–1.03	0.005
Bicarbonate, mEq/L	1.00	1.00–1.00	0.942	0.97	0.97–0.98	<0.001	0.97	0.97–0.98	<0.001
Calcium, mg/dL	1.12	1.10–1.14	<0.001	1.04	1.02–1.06	0.001	1.06	1.03–1.09	<0.001
Creatinine, mg/dL	0.91	0.90–0.91	<0.001	1.01	1.00–1.01	0.075	1.00	1.00–1.01	0.337
Ferritin, per 500 ng/mL	1.26	1.24–1.28	<0.001	1.22	1.20–1.24	<0.001	1.03	1.01–1.05	0.003
Folate, per 5 ng/mL	1.14	1.09–1.19	<0.001	1.05	1.00–1.10	0.057	1.06	1.00–1.11	0.036
Hemoglobin, g/dL	1.05	1.04–1.06	<0.001	1.03	1.02–1.04	<0.001	1.00	0.99–1.02	0.489
iPTH, per 250 pg/mL	0.93	0.92–0.94	<0.001	1.02	1.01–1.03	<0.001	1.02	1.01–1.04	<0.001
Iron, μg/dL	1.02	1.01–1.02	<0.001	1.02	1.02–1.02	<0.001	1.01	1.01–1.02	<0.001
Iron saturation, %	1.05	1.04–1.05	<0.001	1.05	1.05–1.05	<0.001	1.05	1.05–1.05	<0.001
KRU, mL/min	0.96	0.95–0.97	<0.001	0.96	0.95–0.97	<0.001	0.97	0.96–0.97	<0.001
LDH, per 100 U/L	1.09	1.07–1.11	<0.001	1.23	1.21–1.25	<0.001	1.18	1.15–1.20	<0.001
Lymphocyte, per 10% of WBC	0.91	0.90–0.93	<0.001	1.04	1.02–1.06	<0.001	0.98	0.96–0.99	0.009
nPCR, g/kg/d	0.90	0.85–0.95	<0.001	0.89	0.84–0.95	<0.001	0.85	0.79–0.92	<0.001
Phosphorus, mg/dL	0.89	0.88–0.90	<0.001	1.00	0.99–1.01	0.732	1.00	0.99–1.02	0.748
RDW, %	0.99	0.98–0.99	<0.001	0.96	0.95–0.96	<0.001	0.94	0.93–0.94	<0.001
Reticulocyte, %	1.00	0.97–1.04	0.751	1.02	0.99–1.06	0.177	1.01	0.98–1.05	0.471
spKt/V, per 1 increment	1.59	1.53–1.65	<0.001	1.15	1.10–1.20	<0.001	1.05	1.01–1.10	0.026

Table 2. (continued)

	Unadjusted			Case-mix			Case-mix + MICS		
	OR	95% CI	*p* value	OR	95% CI	*p* value	OR	95% CI	*p* value
TIBC, per 100 μg/dL	0.79	0.77–0.81	<0.001	0.82	0.80–0.84	<0.001	1.09	1.05–1.13	<0.001
WBC count, per 10 $\times 10^3$/μL	1.00	0.96–1.05	0.991	0.89	0.85–0.93	<0.001	1.01	0.96–1.06	0.816
Medications									
ESA use, per 10,000 U	1.00	0.99–1.01	0.543	1.00	0.99–1.01	0.919	1.00	0.99–1.01	0.625
IV iron, per 500 mg	0.91	0.91–0.92	<0.001	0.92	0.91–0.93	<0.001	1.00	0.99–1.01	0.616

Please note: In logistic regression analyses, models were restricted to patients that had the risk factor of interest, and were adjusted for all other components in the multilevel adjustment minus the exposure.

BMI, body mass index; ASHD, arteriosclerotic heart disease; CBVD, cerebrovascular disease; CHF, congestive heart failure ; COPD, chronic obstructive pulmonary disease; Other CD, other cardiac disease; CVC, central venous catheters; AVF, arteriovenous fistula; AVG, arteriovenous graft; ALP, alkaline phosphatase; B12, vitamin B12; iPTH, intact parathyroid hormone; KRU, residual renal urea clearance; LDH, lactic acid dehydrogenase; nPCR, normalized protein catabolic rate; RDW, red cell distribution width; spKt/V, single-pooled Kt/V; TIBC, total iron binding capacity; WBC, white blood cell count, ESA, erythropoiesis stimulating agent; IV, intravenous. MCV, mean corpuscular volume.

Infectious Mortality

Patterns for infectious mortality were less consistent than those for all-cause or cardiovascular mortality. The highest group of baseline MCV was associated with a higher risk of infectious mortality across all levels of adjustment (Fig. 1e, online suppl. Table S8), with an 18% increased risk of infectious mortality for MCV 100+ fL compared to the referent (HR 1.18, 95% CI 1.02–1.38) in the fully adjusted model. Associations of lower baseline MCV with lower infectious mortality risk were found but only persisted after all levels of adjustment for MCV groups 86–<88 and 88–<90. One group above the referent, MCV 94–<96, also had reduced risk of infectious mortality. Competing risk regression subHRs (online suppl. Table S10) showed that after accounting for noninfectious mortality as a competing event, the same 3 MCV groups maintained reduced infectious mortality risk, and the highest group maintained increased risk.

In time-varying analysis (Fig. 1f, online suppl. Table S9), no association was observed between lower levels of MCV and infectious mortality, but the 2 highest MCV levels were associated with higher mortality.

Restricted cubic splines for baseline (online suppl. Fig. S4g–i) and time-varying (online suppl. Fig. S5g–i) infectious mortality HRs did not suggest the presence of nonlinear relationships between MCV and infectious mortality.

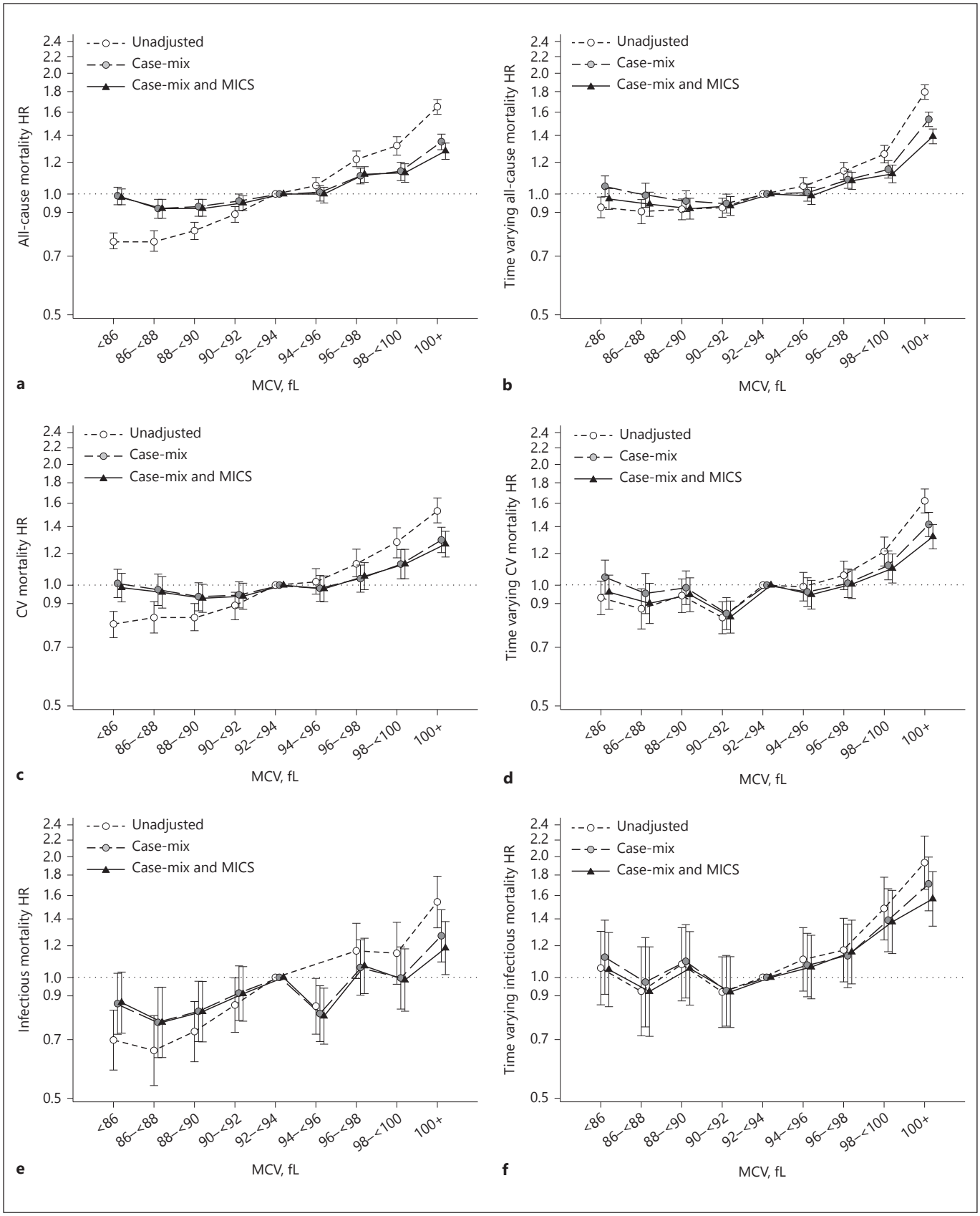
Subgroup Analyses

MCV above or equal to the median (≥93 fL) versus below the median (<93 fL) was consistently associated with higher all-cause, cardiovascular, and infectious mortality across substrata (Fig. 2, online suppl. Table S11). Associations of higher MCV with all-cause mortality were stronger for patients who had diabetes or who were receiving a lower dialysis dose. Dialysis dose also similarly modified the association of higher MCV with cardiovascular mortality.

For all-cause mortality, significant interactions between MCV ≥93 fL and subgroups were observed for race, BMI, diabetes, and dialysis dose (online suppl. Table S12). For cardiovascular mortality, interactions were observed for CHF and dialysis dose subgroups. No significant interactions were observed for infectious mortality.

Discussion

In a large nationally representative cohort of 109,501 adult incident HD patients, our primary finding was a robust and consistent relationship between higher MCV with higher risk of all-cause, cardiovascular, and infectious mortality in both baseline and time-varying models, independent of malnutrition and inflammatory status. Patients with higher MCV tended to be older, female, alcoholic, non-diabetic, of non-Hispanic White race, and had elevated markers of poor nutrition, namely lower



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baseline albumin, nPCR, phosphorus concentrations, serum creatinine, and BMI. Furthermore, KRU was lower in patients with higher MCV levels.

Macrocytosis may be a surrogate for an end-stage malnutrition-related poor health condition. While hypoalbuminemia is associated with malnutrition and inflammation [15], lower nPCR and phosphorus may be indicative of low protein intake [16, 17]. Lower serum creatinine correlates with a lower muscle mass [18, 19] in dialysis patients and declining residual kidney function may be a

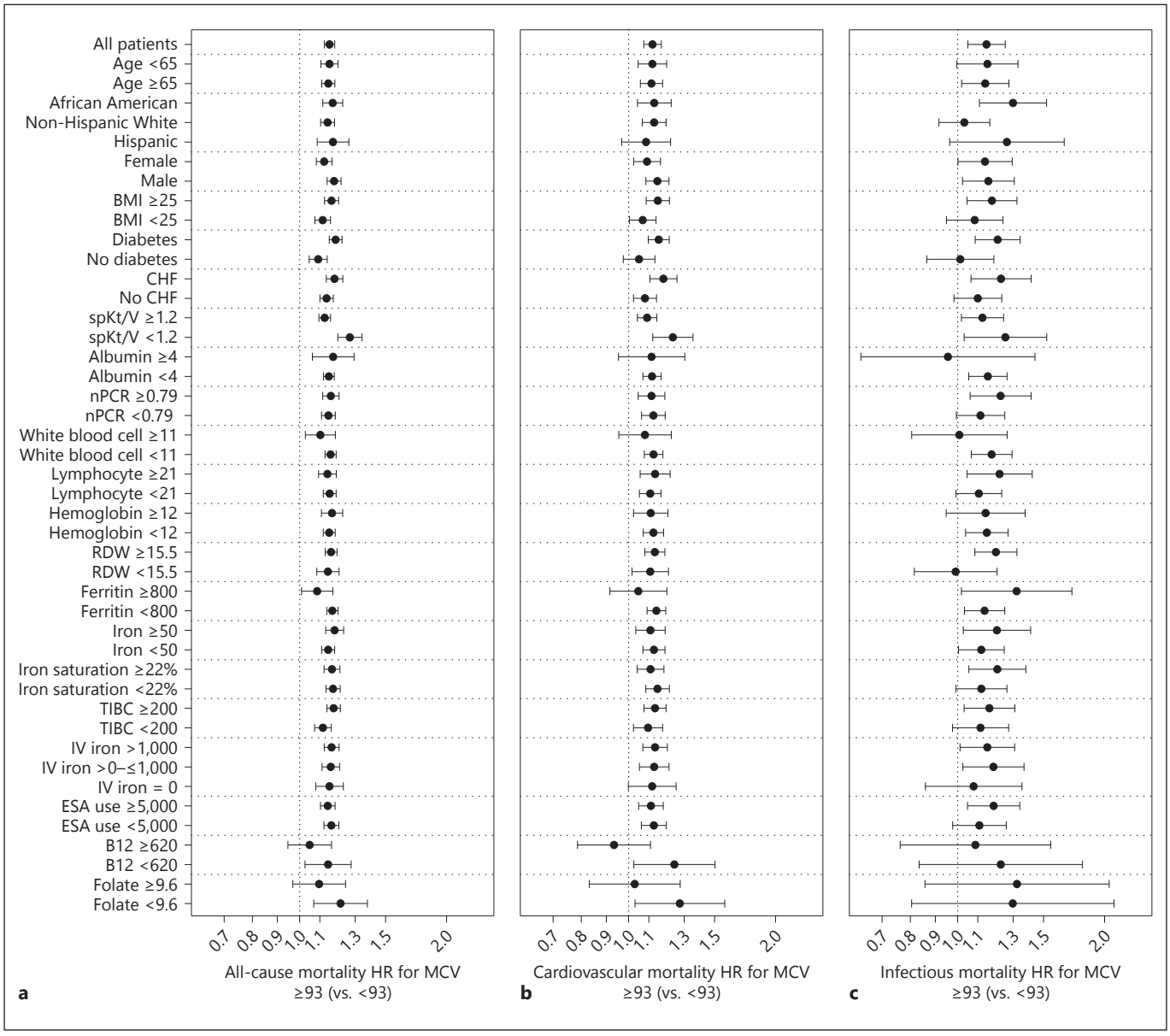

Fig. 2. Subgroup analysis of fully adjusted (case-mix plus MICS) (**a**) all-cause, (**b**) cardiovascular, and (**c**) infectious mortality HRs (and 95% CI error bars) of high MCV (MCV ≥93 fL) vs. low MCV (MCV <93 fL). HR, hazard ratio; MCV, mean corpuscular volume; BMI, body mass index; CHF, congestive heart failure; spKt/V, single-pooled Kt/V; nPCR, normalized protein catabolic rate; RDW, red cell distribution width; TIBC, total iron binding capacity; IV, intravenous; ESA, erythropoiesis stimulating agent; B12, vitamin B12.

Fig. 1. Baseline (**a**) and time-varying (**b**) all-cause, baseline (**c**) and time-varying (**d**) cardiovascular, baseline (**e**) and time-varying (**f**) infectious mortality HRs (and 95% CI error bars) by MCV levels across 3 levels of multivariable adjustment. HR, hazard ratio; MCV, mean corpuscular volume; MICS, malnutrition-inflammation complex syndrome.

factor in malnutrition and inflammation [20]. In fact, protein-energy wasting and inflammation are commonly found in dialysis patients [21, 22]. It has been postulated that malnutrition could lead to a change in osmotic pressure contributing to erythrocyte swelling and a higher MCV [11]. Malnutrition may partially confound the relationship between MCV and mortality, but the association of higher MCV with mortality persisted after MICS adjustment. Additionally, adjusting for residual kidney function in a subset of patients did not remove the association. Therefore, we conclude that malnutrition alone cannot fully explain the association between high MCV and mortality.

Associations between high MCV and B12 and folic acid deficiencies have been well established [6, 23, 24]. Soohoo et al. [25] studied B12- and folate-mortality associations using the same database as was used in this study. They concluded that higher B12 (B12 ≥550 pg/mL) and lower folate (folate <6.2 ng/mL) were associated with higher all-cause mortality. However, in additional analysis including only a subset of our analytical cohort, we found that high MCV was associated with higher all-cause mortality even after adjusting for folate and B12.

Another potential source of residual confounding is the impact of ESA therapy. ESA can lead to an increase in the reticulocyte population [12], which can thereby lead to increased MCV. A previous study described an association between MCV >102 fL and higher darbepoetin to hemoglobin ratio in maintenance HD patients [12], while in another study the MCV-mortality association was not significantly altered after adjustment for medication therapy including ESA [11]. Even though we adjusted for ESA in our baseline and time-varying models, one can argue that this may not account for the time to effect of ESA, since the ESA dose and MCV levels were measured in the same patient quarter. However, the trajectory model of mean MCV over 20 patient quarters stratified by baseline MCV group was not altered by ESA and IV iron adjustment.

Another potential explanation for the association between elevated MCV and mortality may be linked to the molecular aging process. In our study, older age was associated with higher MCV, which is consistent with other studies [26]. Relationships of leukocyte telomere length with heart disease, cancer, infections, and overall mortality have been previously described [27–29]. With every division, DNA polymerase cannot fully replicate the 3' end of a DNA strand; thus the telomere length becomes shorter and eventually reaches a critical length that can lead to cell death [27]. Kozlitina and Garcia [30] measured the length of genomic DNA isolated from circulating leukocytes in 3,157 subjects that were enrolled in the Dallas Heart Study 2. Using a multiple regression model they found that shorter telomeres were significantly associated with larger MCV. Furthermore, shorter telomeres may be related to infectious diseases [31, 32]. In our analysis, associations of higher MCV with mortality were only slightly attenuated after adjustment for age; however, we were unable to account for differing telomere length.

Furthermore, RBC metabolism and homeostasis strongly affect the antioxidant properties of the whole body, and alteration of the erythrocyte membrane may result in a reduction of its antioxidant capacity [9, 33, 34]. Solak et al. [34] showed that MCV was associated with endothelial dysfunction, independent of inflammation, suggesting that the harmful impact of higher MCV level on endothelial function may be a consequence of the compromised antioxidant potential of macrocytic erythrocytes. Interestingly, the overproduction of reactive oxygen species may play a causative role in the development and progression of cardiovascular disease [9].

We were unable to account for these factors in our cohort analysis, and thus recommend future studies with the ability to capture data on oxidative stress to explore this possibility. The precise mechanisms by which MCV and mortality are related are yet to be determined.

Strengths of this study include the large sample size, thorough adjustment for common markers of malnutrition and inflammation, and refined categories of MCV that allowed us to examine non-linear relationships. Limitations include the possibility of residual confounding and our inability to infer causality due to the retrospective observational study design. We also lacked data on certain confounders, namely measures of C-reactive protein, reactive oxygen species, other inflammatory markers, the number of blood transfusions, and medications known to induce macrocytosis. Moreover, we cannot provide any information on pre-ESRD treatment such as ESA dose or IV iron, which might confound the baseline MCV-mortality association. For our baseline models, monthly cumulative IV iron and weekly median ESA use were documented after dialysis initiation over a 90-day period, during the same time frame as the baseline MCV measurement.

In conclusion, in a large national cohort of incident HD patients in the United States, higher MCV levels were associated with higher risk of all-cause, cardiovascular,

and infectious mortality. Further studies are needed to understand the pathophysiology underlying this relationship, and the potential advanced utility of evaluating MCV level in clinical practice.

Acknowledgment

We thank DaVita Clinical Research® (DCR) for providing statistically de-identified data used in this study.

Disclosure Statement

K.K.-Z. has received honoraria and/or support from Abbott, Abbvie, Alexion, Amgen, American Society of Nephrology, AstraZeneca, AVEO, Chugai, DaVita, Fresenius, Genetech, Haymarket Media, Hospira, Kabi, Keryx, National Institutes of Health, National Kidney Foundation, Relypsa, Resverlogix, Sanofi, Shire, Vifor, ZS-Pharma. C.P.K. has received honoraria from Sanofi-Aventis, Relypsa and ZS Pharma.

Funding Sources

K.K.-Z. is supported by NIH (NIDDK) grants K24-DK091419, and philanthropic grants from Mr. Harold Simmons, Mr. Louis Chang, Joseph Lee, and AVEO, Inc.

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